Cancer Biology

Chapter 18

Eric J. Hall., Amato Giaccia,

Radiobiology for the Radiologist

Introduction

- Tissue homeostasis depends on the regulated cell division and self-elimination (programmed cell death) of each of its constituent members except its stem cells
- A tumor arises as a result of uncontrolled cell division and failure for self-elimination
- Alterations in three groups of genes are responsible for the deregulated control mechanisms that are the hallmarks of cancer cells: proto-oncogenes, tumor-supressor genes, and DNA stability genes

Proto-oncogenes

- Proto-oncogenes are components of signaling networks that act as positive growth regulators in response to mitogens, cytokines, and cell-to-cell contact
- A gain-of-function mutation in only one copy of a protooncogene results in a dominantly acting oncogene that often fails to respond to extracellular signals

Tumor-suppressor genes

- Tumor-suppressor genes are also components of the same signaling networks as proto-oncogenes, except that they act as negative growth regulators
- They modulate proliferation and survival by antagonizing the biochemical functions of proto-oncogenes or responding to unchecked growth signals
- In contrast to oncogenes, inactivation of both copies of tumor-suppressor genes is required for loss of function in most cases

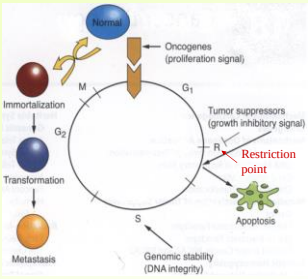
DNA stability genes

- DNA stability genes form a class of genes involved in both monitoring and maintaining the integrity of DNA
- Loss of these genes results in defective sensing of DNA lesions as well as improper repair of the damaged template
- Loss of function results in accumulation of mutations

Mechanisms of carcinogenesis

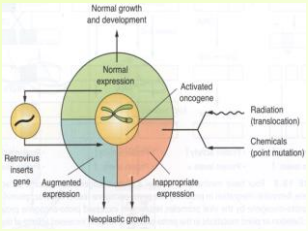
- A single genetic alteration that leads to the activation of an oncogene or loss of a tumor suppressor gene does not lead to the formation of a solid tumor or metastasis
- Most tumors contain heterogeneous populations of cells that differ in their ability to repopulate the tumor or form metastases
- The malignant progression from normal tissue to tumor to metastasis occurs in a number of discrete "steps" over a period of time

Mechanisms of carcinogenesis



- Malignant transformation results from mutations in three groups of genes:
  - gain-of-function mutations that activate oncogenes
  - loss-of-function mutations that inactivate tumor suppressor genes
  - loss of activity of DNA stability (e.g., repair) genes that increases the probability for genomic instability

Oncogenes



- First discovered from studies of retroviruses that cause cancer in animals (Rous, early 1900s)
  - Cell-free extracts derived from chicken sarcomas could cause sarcoma in healthy chickens
- Only a certain portion of the viral genome was identified as needed for malignant transformation (proto-oncogene)
- Either gene mutation or over-expression

Mechanisms of oncogene activation

- Oncogenes are mutant or over-expressed forms of normal cellular genes (proto-oncogenes); the alteration can be produced by various agents
- It is a dominant gene, mutation in only one copy leads to its activation
- At least four mechanisms exist:
  - Retroviral integration of proto-oncogene sequences in retroviral genomes through recombination
  - DNA mutation of regulatory sites
  - Gene amplification
  - Chromosome rearrangement

Mechanisms of oncogene activation

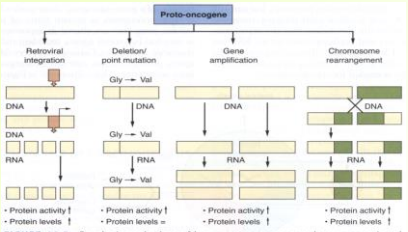


FIGURE 18.3 Four basic mechanisms of how a proto-oncogene can become an activated oncogene. Retroviral integration in proximity to a proto-oncogene results in transcriptional control of the proto-oncogene by the viral promoter, resulting in increased proto-oncogene protein and activity. Deletion or point mutations in the proto-oncogene result in increased activity of the proto-oncogene without necessarily changing transcription or protein levels of the proto-oncogene. Increased copy number of a proto-oncogene by gene amplification results in increased transcription, protein levels, and activity of the proto-oncogene. Chromosome translocation results in an altered proto-oncogene product that can have increased transcription, protein levels, and activity. All of these alterations in a proto-oncogene that occur at the DNA level manifest themselves at the protein level as increased activity and, in some cases, increased protein levels.

Chromosome translocation

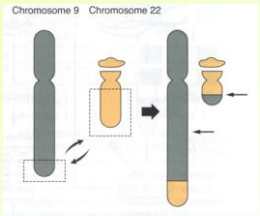


FIGURE 18.5 A symmetric translocation between chromosomes 9 and 22 brings together the *bcl* and *abl* genes to form a fusion gene associated with over 90% of cases of chronic myelogenous leukemia (CML).

- The first real breakthrough in identifying tumor-specific chromosome alterations occurred in the late 1950s when Dr. Peter Nowell found a consistent shortened version of chromosome 22 in individuals afflicted with chronic myelogenous leukemia (CML)

Oncogene activation

TABLE 18.1 Examples of Chromosomal Changes Leading to Oncogene Activation and Their Associated Murine or Human Malignancies*

Oncogene	Chromosomal Change	Cancer
int-1	Proviral insertion	Murine breast carcinoma
int-2	Proviral insertion	Murine breast carcinoma
pim-1	Proviral insertion	Murine T-cell lymphoma
N-*ras*	Point mutation (1)	Melanoma
K-*ras*	Point mutation (12)	Pancreas carcinoma
H-*ras*	Point mutation (11)	Colon carcinoma
neu	Point mutation (17)	Neuroblastoma
N-*myc*	Gene amplification (8)	Neuroblastoma
L-*myc*	Gene amplification (8)	Lung carcinoma
neu	Gene amplification (17)	Breast carcinoma
EGFR	Gene amplification (7)	Squamous cell carcinoma
bcr-abl	Translocation (9–22)	Chronic myelogenous leukemia
c-myc	Translocation (8–14)	Burkitt lymphoma
c-myc	Translocation (2–8)	Burkitt lymphoma
c-myc	Translocation (8–22)	Burkitt lymphoma
bcl-2	Translocation (14–18)	Diffuse large B-cell lymphoma

*Human oncogenes activated by retroviruses have not yet been found in human malignancies, only murine cancers.

Tumor-suppressor genes

- Another class of genes, anti-oncogenes
- Recessive gene, both copies of tumor-suppressor gene have to be inactivated in order to loose function of suppressing malignant transformation
- First discovered through family history studies of patients with hereditary cancers, such as retinoblastoma (*Rb* gene) or Li-Fraumeni syndrome (*p53* gene)

Retinoblastoma studies

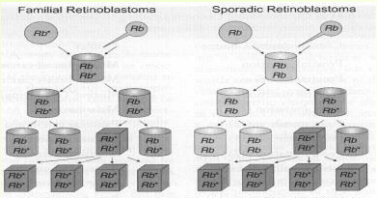


FIGURE 18.6 *Rb* mutations in familial and sporadic retinoblastoma. In familial retinoblastoma, one normal allele (*Rb*) and one mutated allele (*Rb**) are inherited from either parent, resulting in a heterozygous individual containing *Rb/Rb** retinal cells. Subsequent mutation in any retinal cell inactivates the remaining normal *Rb* allele, leading to loss of growth control and expansion of the homozygous mutant *Rb*/Rb** retinal cells that leads to retinoblastoma. In sporadic retinoblastoma, two normal *Rb* alleles are inherited from each parent. First, a mutation inactivates one copy, resulting in heterozygous *Rb/Rb** retinal cells. A subsequent mutation within the same retinal cell inactivates the remaining copy of normal *Rb*, leading to loss of growth control and expansion of homozygous *Rb*/Rb** retinal cells that leads to retinoblastoma. (Illustrating the concepts proposed by Knudson AG. Mutation and cancer: statistical study of retinoblastoma. *Proc Natl*

Li-Fraumeni syndrome studies

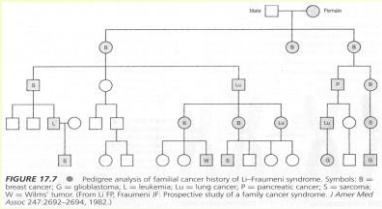


FIGURE 17.7 Pedigree analysis of familial cancer history of Li–Fraumeni syndrome. Symbols: B = breast cancer; G = glioblastoma; L = leukemia; Lu = lung cancer; P = pancreatic cancer; S = sarcoma; W = Wilms' tumor. (From Li FP, Fraumeni JF: Prospective study of a family cancer syndrome. *J Amer Med Assoc* 247:2692–2694, 1982.)

- Extremely rare syndrome, predisposes to development of multiple cancers by young adulthood
- Led to discovery of *p53* gene, its suppression results in a number of tumors

Tumor-suppressor genes

TABLE 18.2 Examples of Cancer Predisposition Genes and Their Associated Syndromes

Tumor Suppressor Gene	Syndrome	Tumor
Rb	Retinoblastoma	Retinoblastoma
WT1	Familial Wilms tumor	Wilms tumor
NF1	Neurofibromatosis type 1	Neurofibroma, sarcoma
NF2	Neurofibromatosis type 2	Schwannoma, meningioma
APC	Familial adenomatosis polyposis	Tumor of colon, stomach, and intestine
p53	Li–Fraumeni syndrome	Breast, lung, brain tumors, sarcoma
VHL	von Hippel-Lindau disease	Tumor of kidney, adrenal
E-CAD	Familial gastric cancer	Tumor of stomach, breast
PTCH	Gorlin syndrome	Basal cell carcinoma
PTEN	Cowden syndrome	Hamartoma
MEN1	Multiple endocrine neoplasia	Tumor of pituitary, pancreas, and parathyroid

Tumor-suppressor genes

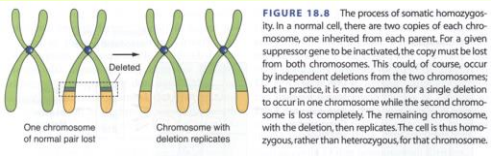


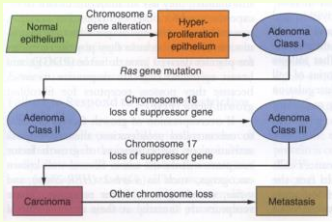
FIGURE 18.8 The process of somatic homozygosity. In a normal cell, there are two copies of each chromosome, one inherited from each parent. For a given suppressor gene to be inactivated, the copy must be lost from both chromosomes. This could, of course, occur by independent deletions from the two chromosomes; but in practice, it is more common for a single deletion to occur in one chromosome while the second chromosome is lost completely. The remaining chromosome, with the deletion, then replicates. The cell is thus homozygous, rather than heterozygous, for that chromosome.

- Often tumor-suppressor gene is lost through somatic homozygosity: one chromosome of a pair is lost, a deletion occurs in the remaining chromosome; the chromosome with the deletion replicates
- This process has been documented for a number of tumors

The multi-step nature of cancer

- Carcinogenesis is a multi-step process: a number of distinct events that may be separated in time have to occur
- Genetic analysis of cells from solid tumors suggests alterations, mutations, or deletions in multiple signaling genes, either oncogenes or suppressor genes. For example, 6 to 12 mutations have been suggested for the formation of a carcinoma
- The following stages can be identified in tumor development: *initiation, promotion, and progression*

The multi-step nature of cancer



- A model proposed for colorectal cancer correlates a series of chromosomal and molecular events with the changes in the histopathology of normal epithelium during the multistage formation of colorectal cancer and metastatic carcinoma

The multi-step nature of cancer

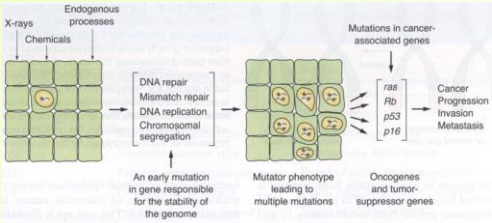


FIGURE 18.10 Illustrating the multistep nature of carcinogenesis and the concept of the mutator phenotype. The first step in carcinogenesis by radiation or any other agent may be a mutation in one of the gene families responsible for the stability of the genome. This may be a DNA repair gene, an MMR gene, or a gene in a family as yet unidentified. This leads to the mutator phenotype, with multiple mutations possible in both oncogenes and tumor suppressor genes. This then leads to a series of steps that result in an invasive metastatic cancer. Not all the same mutations need to be present in every case.

Functions of oncogenes and tumor-suppressor genes

- Several categories of cell functions are perturbed by mutations in oncogenes and tumor-suppressor genes
- Mostly these are functions related to regulation of proliferation, growth-restriction and apoptosis signals
- Combination in deregulations of these functions lead to tumor initiation, invasion and metastasis

Deregulated proliferation

- Normal cells rely on extracellural growth signals; typically one cell secretes a mitogenic signal to stimulate the proliferation of another cell type
- Signal is initiated at the cell membrane (receptors) and is transduced to the nucleus via a cascade of proteins affecting regulatory functions
- In contrast to untransformed cells, transformed cells become autonomous in regulating their growth by responding to the mitogenic signals they themselves produce

Deregulated proliferation

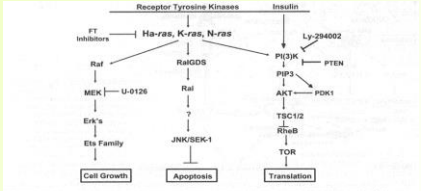


FIGURE 17.11 The Ras family (H-Ras, K-Ras and N-Ras) are proteins that act as GDP/GTP-regulated binary switches that reside at the inner surface of the plasma membrane and act to relay extracellular ligand-stimulated signals to cytoplasmic signaling cascades. However, oncogenic forms of Ras lose their responsiveness to growth receptor tyrosine-kinases and constitutively activate cascades of serine–threonine kinases that control cell proliferation, inhibit apoptosis, and increase protein translation. These pathways are depicted in a linear fashion, but are more complex and are regulated by positive and negative feedback loops. This illustration depicts three direct effectors of Ras that have been shown to mediate these events. Ras stimulates proliferation by activating the Raf–MEK–Erk pathway that phosphorylates and activate the Ets transcription factor that leads to increased levels of cyclin D_1, mRNA, and protein. Increased levels of cyclin D_1 result in increased Rb phosphorylation and transition of cells from G_1 to S phase of the cell cycle. A second direct effector of Ras, RalGDS, is a guanine nucleotide exchange factor that activates Ral A and B GTPases and promotes tumor cell survival through the JNK/SEK (Jun kinase/stress kinase) pathway. The third major pathway that Ras directly regulates is the PI3K pathway. This pathway has long been known to be important in regulating cell growth through insulin signaling. Like Raf and RalGDS, oncogenic forms of Ras increase PI3K activity that leads to increased phosphorylation of the lipid kinase PIP3, which in turn inhibits the negative regulation of TOR by TSC1/2 and RheB. Increased TOR leads to increased translational initiation of mRNAs that possess a structured 5′ region known as a "cap." This is an oversimplified illustration, because there are at least three other signaling pathways that lie downstream of Ras. The Ras pathway can be disrupted using pharmacologic inhibitors of farnesyltransferases (FT), PI(3)K (Ly-294002), or MEK (U-0126). (Adapted from Marianne Powell with permission.)

Failure to respond to growth-restrictive signals

- The oncogenic activation stimulates the cell into the S phase, where it duplicates its genetic material before cell division
- Inactive tumor suppressor genes fail to enforce the restriction point in G1 phase, allowing cells to escape extracellular anti-proliferative signals

Failure to commit suicide (apoptosis)

- Two major pathways that mediate cell death originate either from the cell membrane or from the mitochondria
- The signals transmitted by each pathway results in the activation of intracellular proteins, termed caspases, that cleave a diverse number of proteins at specific sites
- Cell lines deficient in Caspases 3 and 9 exhibit substantially reduced levels of apoptosis during development and in response to stress-inducing stimuli
- Tumor-suppressor gene *p53* in an important modulator of oncogene-induced apoptosis

Oncogene *myc*

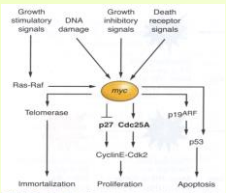
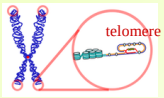


FIGURE 18.14 The *myc* oncogene can stimulate cell proliferation and primes the cell for apoptotic cell death. Depending on the cellular context, *myc* can stimulate proliferation through the activation of cyclin E–cdk2, which phosphorylates *pRb* and drives cells into S phase in response to mitogenic signals, and *myc* can prime cells for apoptosis by increasing the levels of p53 through ARF (*p19*). Also, *myc* can promote the inactivation of the cyclin–cdk inhibitor *p27* as well as increase Cdc25A phosphastase activity. Also, *myc* can induce immortalization through regulating telomerase activity. Therefore, *myc* is the prototypical example of how one oncogene can affect cell proliferation, cell death, and immortalization.

- *myc* codes for a protein that binds to the DNA of other genes and is therefore a transcription factor
- When a gene like *myc* is altered to cause cancer, the cancerous version of the gene is called an oncogene
- The healthy version of the gene that it is derived from is called a proto-oncogene

Escaping senescence



A telomere is a region of DNA (repeat sequence of TTAGGG) at each end of a chromatid, protecting it from deterioration and fusion with other chromatids

- Each time a normal somatic cell divides, the terminal end of the telomere is lost; successive divisions lead to progressive shortening, and after 40 to 60 divisions, vital DNA sequences are lost. At this point, the cell cannot divide further and undergoes senescence
- Cancer cells avoid this process of aging by activating the enzyme telomerase, which offsets the degradation of telomeres at successive cell divisions; thus becoming immortal
- Mutation in tumor-suppressor gene p53 is involved

Angiogenesis

- Angiogenesis, the recruitment of new blood vessels to regions of chronically low blood supply, is essential for the progression of solid tumors to malignancy
- A number of proangiogenic growth factors have been identified, VEGF was the first growth factor isolated that could stimulate proliferation and migration of blood vessel cell lining
- Studies have shown that blocking the binding of VEGF to its receptor inhibits tumor angiogenesis and tumor growth. These findings have led to the development of new antibody approaches for antiangiogenesis therapy for clinical use

Invasion and metastasis

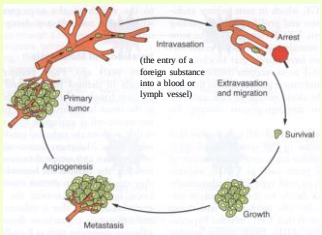


FIGURE 18.16 Critical steps in the metastatic process. Tumor cells acquire the ability to disrupt the extracellular matrix of the basement membrane, allowing them to intravasate into local blood or lymph vessels. To form metastases at remote locations, they must migrate and extravasate through the host tissue, blood, or lymph supply and survive and proliferate in the new soil. All of these processes are depicted in this figure. (Adapted from Le Q, Denko NC, Giaccia AJ. Hypoxic gene expression and metastasis. *Cancer and Metastasis Rev.* 2004;23:293–310, with permission.)

- The genetic circuits that regulate metastasis remain mainly undiscovered
- Decreased expression or impaired function of adhesion molecules, and regulatory alterations in chemical agents for digestion of extracellular matrix (*ECM*) have been implicated

Gatekeepers and caretakers

- Most tumor-suppressor genes can be broadly divided into two classes that have been called "gatekeepers" and "caretakers"
- *Gatekeepers* are genes that directly regulate the growth of tumors by inhibiting cell division or promoting cell death, rate limiting for tumor growth. Both alleles (maternal and paternal) must be lost or inactivated for a tumor to develop. The identity of gatekeepers varies with each tissue
- Inactivation of *caretaker* genes does not directly promote the growth of tumors, but leads instead to genomic instability that only indirectly promotes growth by causing an increase in mutation rate. The targets of the accelerated mutation rate that occurs in cells with defective caretakers are the gatekeeper tumor-suppressor genes, oncogenes, or both

Mismatch repair genes

- Mismatch repair (MR) genes are responsible for correction of errors of DNA replication and recombination that result in mispaired (but undamaged) nucleotides
- Their primary function is to scan the genome as it replicates and spot errors of mismatch
- Mutations in MR genes were found responsible for the mutator phenotype associated with a predisposition for hereditary nonpolyposis colon cancer (HNPCC) and possibly other familial cancers

Heritable syndromes that affect radiosensitivity

- Ataxia Telangiectasia (lack of voluntary coordination of muscle movements, overdeveloped blood vessels in ocular area, immune deficiency, high incidence of cancers) is associated with a hypersensitive skin reaction to ionizing radiation and DNA breaking agents but not to ultraviolet light
- AT-like disorder (same clinical features, milder symptoms)
- Nijmegen Breakage syndrome (by microcephaly, a distinct facial appearance, short stature, immune deficiency, and a strong predisposition to lymphoid malignancy) - very high sensitivity to ionizing radiation
- All three are autosomal recessive diseases, lack DNA-damage checkpoints, but different gene mutations are responsible

Heritable syndromes that affect genomic instability

- Seckel syndrome (microcephaly and abnormal development)
- Fanconi anemia (hematological abnormalities, median age ~30)
- Syndroms associated with decrease in RecQ gene expression:
  - Bloom syndrome (dwarfism, high sensitivity to light)
  - Werner syndrome (premature aging)
  - Rothmund-Thompson (growth deficiency, photosensitivity, early graying and hair loss)
- Abnormal DNA is accumulated through S phase

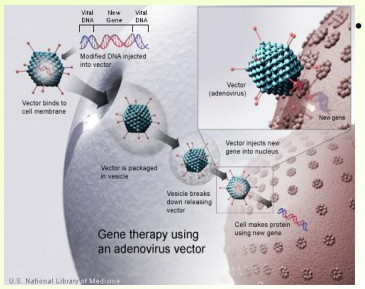
Radiation-induced signal transduction

- Ionizing radiation can regulate the expression of early-response genes, resulting in the stimulation of signal transduction pathways and activation of transcription factors
- It may also enhance the response of the cell to radiation in terms of repair and cell-cycle arrest; and provide a mechanism for secondary stimulation of various late-response genes
- Understanding of these defense mechanisms can help exploiting them for treatment of cancer

Approaches to gene therapy

- **Gene therapy** is the insertion of genes into an individual's cells and tissues to treat a disease: mutant allele is replaced with a functional one
- There are al least 6 different approaches
  - Suicide-gene therapy
  - Cytotoxic virus targeted to p53-deficient cells
  - Molecular immunology (cancer vaccines)
  - Tumor-suppressor gene therapy
  - Radiation-inducible gene linked to a cytotoxic agent
  - Targeting signal transduction pathways

Gene therapy



- Genes are introduced into tumor cells using viral vectors: retrovirus, adenovirus, and herpesvirus

Suicide-gene therapy

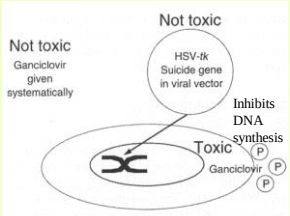


FIGURE 26.1 ● The principle of suicide-gene therapy. The thymidine kinase gene from the herpes simplex virus (HSV-*tk*), contained in a viral vector (adenovirus, so that dividing and nondividing cells can be infected), is injected into the tumor. Ganciclovir is administered systemically. This is a prodrug that is in itself nontoxic. In cells containing the thymidine kinase gene, the prodrug is activated to become a toxic agent.

- Suicide-gene therapy is based on transducing cells with a gene that converts a prodrug into a cytotoxic agent
- There is a substantial bystander effect; that is, more cells are killed than transduced initially
- This therapy has produced growth delay and some cures in animal models
- Because of the limited efficiency of gene delivery, suicide-gene therapy needs to be combined with conventional radiotherapy
- Phase I/I I clinical trials have shown promise

Targeted p-53 deficient cells

- A cytotoxic virus can be constructed that is engineered to replicate and kill only in cells with mutant *p53*
- To the extent that mutant *p53* is a hallmark of cancer, this treatment differentiates between normal cells and cancer cells
- Growth arrest has been observed in model animal tumors and in early clinical trials by targeting mutant *p53*

Molecular immunology (cancer vaccines)

- The approach is to provoke a cellular immune response against the cancer by injecting a vaccine genetically engineered to express immune stimulatory molecules or tumor-specific antigens
- Molecular immunology shows some promise in animal models but is generally only effective against small tumor burdens
- Developing strategy is to combine molecular immunology with suicide-gene therapy

Tumor-suppressor gene therapy

- Tumor-suppressor gene therapy is the replacement, with a correct copy, of the mutated gene that initiates or contributes significantly to the malignant phenotype
- The gene *p53* has received the most attention of any gene because it is so commonly mutated in human cancers
- Phase I/I I clinical trials show some promise in the treatment of non-small-cell lung cancer
- The therapy is limited by a lack of information on the target genes that are essential for maintaining the malignant phenotype and the fact that multiple genetic changes are involved

Radiation-inducible gene linked to a cytotoxic agent

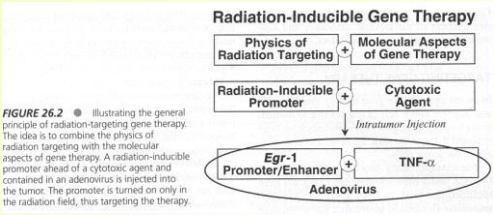


FIGURE 26.2 ● Illustrating the general principle of radiation-targeting gene therapy. The idea is to combine the physics of radiation targeting with the molecular aspects of gene therapy. A radiation-inducible promoter ahead of a cytotoxic agent and contained in an adenovirus is injected into the tumor. The promoter is turned on only in the radiation field, thus targeting the therapy.

- Combination of the physics of radiation-targeting technology with molecular gene therapy

Radiation-inducible gene linked to a cytotoxic agent

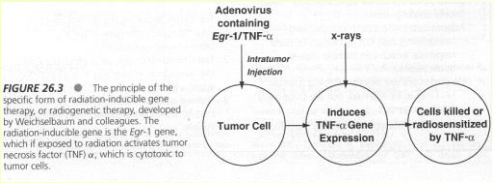


FIGURE 26.3 ● The principle of the specific form of radiation-inducible gene therapy, or radiogenetic therapy, developed by Weichselbaum and colleagues. The radiation-inducible gene is the *Egr*-1 gene, which if exposed to radiation activates tumor necrosis factor (TNF) α, which is cytotoxic to tumor cells.

- There is the potential to use a more radiation-specific promoter gene and a more effective toxic agent
- There is the possibility of including a promoter that is specific for a particular tumor, for example, prostate or breast cancer (in some human cancers, advancing to phase II trials)

Targeting signal transduction pathways

A hallmark of the malignant cell is the dysregulation of growth and signal transduction pathways that often result in resistance to radiotherapy. Several potential targets have been identified:

- –The epidermal growth factor receptor (EGFR) mediates growth regulation in a wide spectrum of human cancers, and tumors expressing high levels of EGFR appear to be radioresistant
- –Raf-1 is a kinase that plays an important role in cell proliferation, differentiation, survival, and angiogenesis and is therefore a prime target for novel cancer therapies
- –NF/cB is a cellular transcription factor that plays a central role in the cellular stress response

Summary

- Development of molecular techniques, such as gene identification and manipulation tools greatly advanced identification of specific genes and understanding of genetic pathways responsible for tumor proliferation
- There is a number of approaches to gene therapy; the winning approach will be a synergistic combination of several treatment modalities